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(54) Title: PRESERVED PHARMACEUTICAL COMPOSITIONS COMPRISING CYCLODEXTRINS

(57) Abstract: A composition comprising a steroid, a cyclodextrin, and a polyhexamethylene biguanide is disclosed herein. Preservatives and methods related thereto, and experimental results suggesting certain advantages related to these compositions, preservatives, and methods are also presented herein.

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PRESERVED PHARMACEUTICAL COMPOSITIONS COMPRISING CYCLODEXTRINS

by

Robert T. Lyons, James Chang, and Chin-Ming Chang

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation-in-part of U.S. Patent Application No. 10/121,076, filed on December 26, 2002; which claims priority under 35 U.S.C. §119(e)(1) to provisional application No. 60/289,337, filed May 7, 2001, both of which are hereby incorporated by reference herein.

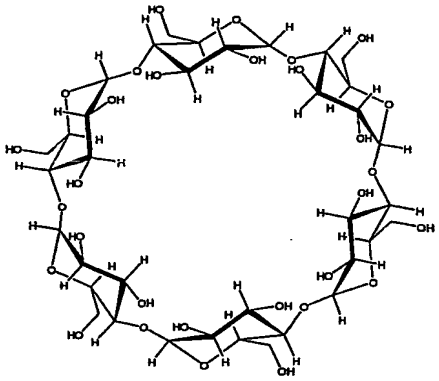
Field of the Invention

The present invention relates to pharmaceutical compositions. In particular, the present invention relates to compositions comprising a steroid and a cyclodextrin.

Background of the Invention

Description of the Related Art

Cyclodextrins are cyclic oligosaccharides containing 6, 7, or 8 glucopyranose units, referred to as α-cyclodextrin (structure depicted below), β-cyclodextrin, or γ-cyclodextrin respectively, which are often used in pharmaceutical formulations.



α-CYCLODEXTRIN

Cyclodextrins have a hydrophilic exterior, which makes them water-soluble, and a hydrophobic interior which forms a cavity. In an aqueous environment, hydrophobic portions of molecules often enter the hydrophobic cavity of cyclodextrin to form inclusion compounds. Although inclusion compounds are often formed between cyclodextrins and hydrophobic molecules, cyclodextrins are also capable of other types of nonbonding interactions with molecules that are not inside the hydrophobic cavity. Cyclodextrins have three free hydroxyl groups for each glucopyranose unit, or 18 hydroxyl groups on α-cyclodextrin, 21 hydroxyl groups on β-cyclodextrin, and 24 hydroxyl groups on γ-cyclodextrin. One or more of these hydroxyl groups can be reacted with any of a number of reagents to form a large variety of cyclodextrin derivatives. Some of the more common derivatives of cyclodextrin are hydroxypropyl ethers, sulfonates, and sulfoalkylethers.

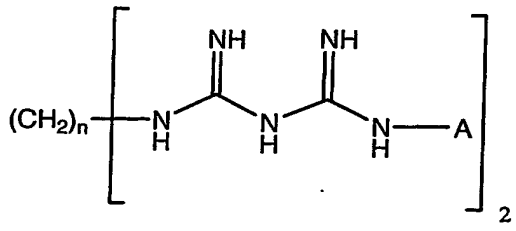
In pharmaceutical formulations, cyclodextrins and cyclodextrin derivatives are often used to improve the solubility of a drug. While inclusion compounds are involved in many cases of enhanced solubility, other interactions between cyclodextrins and insoluble compounds can also improve solubility. As mentioned, the use of cyclodextrins in pharmaceutical compositions is well known in the art. For example, US Patent No. 6,407,079 teaches the use of β-cyclodextrin derivatives to form inclusion compounds that improve the solubility of the drug

Cyclodextrin derivatives have been demonstrated to be useful in solubilizing lipophilic or water-insoluble therapeutic agents or drugs. For example, US 5,472,954 discloses the use of hydroxypropylmethylcellulose and

hydroxypropyl cyclodextrins to solubilize hydrocortisone. The use of cyclodextrin and cyclodextrin derivatives in ophthalmic formulations is also known. For example, EP 0435682 A2 teaches the use of cyclodextrins in ophthalmic compositions with prostaglandins to treat ocular hypertension.

Antimicrobial preservation of cyclodextrin-containing formulations can present special problems. For example, Loftsson et al., Drug Development and Industrial Pharmacy, 18 (13), 1477-1484 (1992), have investigated interactions between several commonly used preservatives and 2-hydroxypropyl-β-cyclodextrin (HPβCD). Loftsson et al. reported that the antimicrobial activity of the preservative can be reduced by the formation of preservative-cyclodextrin inclusion complexes, specifically chlorobutanol, methylparaben, propylparaben, had significantly reduced preservative activity for a number of pathogens, and it was shown that chlorobutanol reduces the solubilizing effects of HPβCD on hydrocortisone, prednisolone, and triamcinolone. However, benzalkonium chloride and chlorhexidine gluconate did possess preservative activity in HPβCD solutions. Additionally, Simpson, FEMS Microbiology Letters, 90, 197-200 (1992), reported that cyclodextrins can inactivate the antimicrobial activity of certain quaternary ammonium compounds. See also, Miyajima et al., Chem. Pharm. Bull., 35(1), 389-393 (1987), regarding the interaction of short-chain alkylammonium salts with cyclodextrins in aqueous solutions, which concluded that α-, β-, and γ-cyclodextrins form complexes with alkylammonium salts having alkyl groups longer than n-butyl, n-hexyl, and n-decyl, respectively.

JP 60149530 A (Takeda Chem. Ind., Ltd.) discloses aqueous compositions of a principal agent and a cyclodextrin where the compositions contain as a preservative a chlorhexidine derivative of the formula



where A is [independently] (un)substituted phenyl; n is 3-9; and the polymethylene chain may be interrupted by an oxygen atom or an aromatic ring.

JP 01016728 A (Santen Seiyaku KK) discloses antiseptic aqueous preparations containing a drug, a cyclodextrin and a cationic surfactant as a preservative. By adding a cyclodextrin or cyclodextrin derivative, cationic surfactants commonly incompatible with certain drugs can be combined. Disclosed cationic surfactants are benzalkonium chloride, benzethonium chloride or chlorohexidine gluconate. Disclosed drugs include sodium hyaluronate, pilocarpine hydrochloride, lysosyme chloride, Na_2 chondroitin sulfate, glycyrrhetinate, pirenoxine, sodium chromoglycate, and dimethylisopropylazulene sodium sulfate.

JP 6016547 A (Wakamoto Pharm. Co. Ltd.) discloses eye drop compositions containing diclofenac sodium and a water soluble cyclodextrin compound. The reference also discloses that these compositions can be preserved using benzalkonium chloride, benzethonium chloride and chlorhexidine gluconate as cationic surfactants; methylparaben, ethylparaben, propylparaben and butylparaben as parabens; and phenylethyl alcohol and benzyl alcohol as alcohols.

U.S. Patent No. 5,998,488 discloses "The ophthalmic composition of the invention contains (1) an antimicrobial preservative having a cationic group, (2) a cyclodextrin, (3) ethylenediaminetetraacetic acid or a salt thereof, and (4) boric acid and/or borax as essential components." This patent also discloses that "The antimicrobial preservative having a cationic group used herein may be selected from well-known antimicrobial preservatives, for example, quaternary ammonium salts such as benzalkonium chloride, benzethonium chloride, cetyldimethylbenzylammonium chloride, domiphen bromide, 3-(trimethoxysilyl)propyldimethyloctadecylammonium chloride, stearyldimethylbenzylammonium chloride, stearyltolylmethyl-ammonium chloride, distearyldimethylammonium chloride, stearylpentaethoxyammonium chloride, cetylpyridinium chloride, cetylpyridinium bromide, and lauroylisoquinolium bromide; and guanidines such as chlorohexidine hydrochloride, chlorohexidine gluconate, dodecylguanidine hydrochloride,

polyhexmethylenebiguanidine hydrochloride, and 6-acetoxy-2,4-dimethylmetadioxane." However, the patent further states "benzalkonium chloride is most effective and preferable."

In citing the foregoing references, and other references cited herein, applications make no admission as to whether any of said references constitutes prior art. Rather, the determination of what constitutes prior art is a legal exercise made on the basis of the dates said references were made available to the public, the authors or inventors of said references, and the effective filing date of the disclosure made herein.

BRIEF DESCRIPTION OF THE INVENTION

A composition comprising a steroid, a cyclodextrin, and polyhexamethylene biguanide (PHMB) is disclosed herein.

A method comprising providing an ophthalmic composition comprising a steroid and cyclodextrin with an effective amount PHMB, wherein said method prevents, attenuates, or reduces the pathogenic contamination of said composition is also disclosed herein.

DETAILED DESCRIPTION OF THE INVENTION

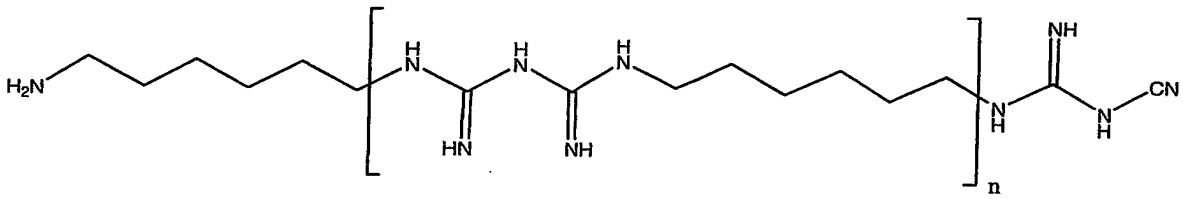
While not intending to be limited or bound in any way by theory, we have surprisingly discovered that PHMB is particularly useful in preserving compositions comprising a steroid and a cyclodextrin. The term "cyclodextrin" as disclosed herein should be interpreted broadly to include the natural cyclodextrins and their derivatives, including the alkylated and hydroxyalkylated derivatives and the branched cyclodextrins. Cyclodextrins and their derivatives which have been previously described as useful for complexation with drugs are of particular interest herein. In addition to α-, β- and γ-cyclodextrin, the ether and mixed ether derivatives and those derivatives bearing sugar residues are of special interest. Especially useful herein are the hydroxyethyl, hydroxypropyl (including 2- and 3-hydroxypropyl) and

dihydroxypropyl ethers, their corresponding mixed ethers and further mixed ethers with methyl or ethyl groups, such as methyl-hydroxyethyl, ethyl-hydroxyethyl and ethyl-hydroxypropyl ethers of α-, β- and γ-cyclodextrin. Hydroxypropyl-β-cyclodextrin and its preparation by propylene oxide addition to β-cyclodextrin, and hydroxyethyl-β-cyclodextrin and its preparation by ethylene oxide addition to β-cyclodextrin, were described in a patent of Gramera et al. (U.S. Pat. No. 3,459,731, issued August 1969) over 20 years ago. Other useful cyclodextrin derivatives are maltosyl, glucosyl and maltotriosyl derivatives of β-and γ-cyclodextrin, which may contain one or more sugar residues, e.g. glucosyl or diglucosyl, maltosyl or dimaltosyl, as well as various mixtures thereof, e.g. a mixture of maltosyl and dimaltosyl derivatives. Other useful cyclodextrin derivatives comprise anionic functional groups such as sulfobutylether derivatives, sulfonates, phosphates, and the like. Specific examples of cyclodextrin derivatives for use herein include hydroxypropyl-β-cyclodextrin, hydroxypropyl-γ-cyclodextrin, sulfobutylether-β-cyclodextrin, and sulfobutylether-γ-cyclodextrin, as well as hydroxyethyl-β-cyclodextrin, hydroxyethyl-γ-cyclodextrin, dihydroxypropyl-β-cyclodextrin, glucosyl-β-cyclodextrin, diglucosyl-β-cyclodextrin, maltosyl-β-cyclodextrin, maltosyl-γ-cyclodextrin, maltotriosyl-β-cyclodextrin, maltotriosyl-γ-cyclodextrin and dimaltosyl-β-cyclodextrin, and mixtures thereof such as maltosyl-β-cyclodextrin/dimaltosyl-β-cyclodextrin. Procedures for preparing such cyclodextrin derivatives are well-known, for example, from Bodor U.S. Pat. No. 5,024,998, dated Jun. 18, 1991, expressly incorporated herein by reference, and references cited therein. Cyclodextrins are used in pharmaceutical compositions for a number of reasons including, but not limited to, solubilizing active ingredients or excipients, stabilizing active ingredients or excipients, modulating bioavailability, reducing side effects and the like.

The amount of cyclodextrin used in the compositions disclosed here is dependent upon the particular situation, and can vary. While not intended to limit the scope of the invention in any way, in many compositions the concentration of cyclodextrin is from 0.1% to 40%. In other compositions, the

cyclodextrin concentration is from 10% to 30%. In some compositions, the cyclodextrin concentration is about 20%.

Polyhexamethylene biguanide (PHMB), also known as polyaminopropyl biguanide and polihexanide has the structure shown below. In the pH range used in ophthalmic compositions, one or more of the nitrogen atoms is protonated, and the compound is thus generally cationic. One commercially available form of PHMB is known by the tradename COSMOCIL® CQ, manufactured by [Avecia, Inc., Wilmington, Delaware], which is sold as a 20% aqueous solution of PHMB HCl having a molecular weight of 2500 ± 300, and an average n (structure) of 10-13. PHMB HCl is the hydrochloride salt of PHMB, where there are n HCl species per molecule.



PHMB

An effective amount of PHMB for the compositions disclosed herein can be readily determined by a person having ordinary skill in the art. This amount can vary, depending upon the particular composition in which it is used. In certain compositions, the concentration of the guanidine-based cationic compound is from about 0.1 ppm to 25 ppm. In other compositions, the concentration is from 1 ppm to 5 ppm. In other compositions, the concentration is from 3 to 5 ppm.

The term "sorbic acid" as used herein, applies to both sorbic acid and sorbate salts. Thus, sodium sorbate, potassium sorbate, ammonium sorbate, or any salt of sorbic acid could be used in the methods and compositions disclosed herein and should be interpreted to mean "sorbic acid" as indicated by the claims herein. It is understood that in an aqueous solution having a pH of 7, sorbic acid, which has a pK_a of 4.76 will be essentially completely deprotonated. Thus, the actual form of sorbic acid in a composition may be

different that that which was added to the composition, and the term "sorbic acid" should be applied as broadly as generally understood in the art in light of these considerations. In a case where a mass-dependent concentration is given for sorbic acid, the concentration is defined as the concentration of the neutral form of sorbic acid, regardless of what form is added, or what form is actually present in the composition. An effective concentration of the sorbic acid can be readily determined by a person of ordinary skill in the art, and can vary. In certain compositions, the concentration of sorbic acid is between 0% and 5%. In other compositions, the concentration of sorbic acid is between 0.05% and 5%. In other compositions, the concentration of sorbic acid is from 0.05% to 1%. Other compositions comprise from 0.05% to 0.4% sorbic acid. Other compositions comprise about 0.6% sorbic acid.

A preservative is an excipient which is effective in preventing, attenuating, or reducing the pathogenic contamination of said composition microbial or pathogenic contamination in an ophthalmic composition. In other words, a preservative might kill pathogens that are present in a composition; prevent the growth of one or more pathogens; attenuate, or reduce, the rate of growth of one or more pathogens; or a combination of these. Standard tests of antimicrobial effectiveness exists for various government organizations including the United States Food and Drug Administration's USP test, and the European Union's Ph Eur-A and Ph Eur-B tests. Tests are often carried out on standard microbial species such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Candida albicans*, and *Aspergillus niger*.

The term "steroid" as used herein has the broadest meaning generally understood by those of ordinary skill in the art. In one embodiment, the steroid is an estrogen; a glucocorticoid; a progestin; a mineralocorticoid; a corticosteroid, such as cortisone, hydrocortisone, prednisone, prednisolone, methylprednisone, triamcinolone, fluoromethalone, dexamethasone, medrysone, betamethasone, loteprednol, fluocinolone, flumethasone, or mometasone; or an androgen such as testosterone, methyltestosterone, or danazol.

In ophthalmic compositions, a chelating agent may be used to enhance preservative effectiveness. Suitable chelating agents are those known in the art,

and, while not intending to be limiting, edetate (EDTA) salts like edetate disodium, edetate calcium disodium, edetate sodium, edetate trisodium, and edetate dipotassium are examples of useful chelating agents. It is understood that EDTA refers to a species having four carboxylic acid functional groups, and that these carboxylic acid groups may be protonated or deprotonated (i.e. in the salt form) depending upon the pH of the composition it is in.

As is known in the art, buffers are commonly used to adjust the pH to a desirable range for ophthalmic use. Generally, a pH of around 5-8 is desired, however, this may need to be adjusted due to considerations such as the stability or solubility of the therapeutically active agent or other excipients. In compositions comprising prednisolone acetate, a pH of from 4 to 6 may help to stabilize the compound. Other prednisolone acetate containing compositions have a pH of from 4.5 to 5.5. Other prednisolone acetate containing compositions have a pH of about 4.5.

Many buffers including salts of inorganic acids such as phosphate, borate, and sulfate are known. When the concentration of a buffer is given, it refers to the total concentration of the buffering species. In other words, if a concentration contains 0.01 M bisulfate and 0.01 M sulfate, the buffer concentration is 0.02 M. Generally, while not intending to be limiting, in an ophthalmic composition, the buffer concentration can be up to about 0.2 M. Some compositions comprise from 0 to 50 mM buffer. Other compositions comprise from 5 to 15 mM buffer. Still other compositions comprise from 0 to 10 mM buffer. Other compositions comprise about 10 mM buffer.

Another commonly used excipient in ophthalmic compositions is a viscosity-enhancing, or a thickening agent. Thickening agents are used for a variety of reasons, ranging from improving the form of the formulation for convenient administration to improving the contact with the eye to improve bioavailability. The viscosity-enhancing agent may comprise a polymer containing hydrophilic groups such as monosaccharides, polysaccharides, ethylene oxide groups, hydroxyl groups, carboxylic acids or other charged functional groups. While not intending to limit the scope of the invention, some examples of useful viscosity-enhancing agents are sodium

carboxymethylcellulose, hydroxypropylmethylcellulose, povidone, polyvinyl alcohol, and polyethylene glycol.

In ophthalmic solutions, tonicity agents often are used to adjust the composition of the formulation to the desired isotonic range. Tonicity agents are well known in the art and some examples include glycerin, mannitol, sorbitol, sodium chloride, and other electrolytes.

Another composition consists essentially of from 0.6 to 1.8% prednisolone acetate, from 10% to 25% hydroxypropyl-γ-cyclodextrin, from 0% to 0.25% hydroxypropylmethylcellulose, from 3 to 10 ppm polyhexamethylene biguanide HCl, from 0.05% to 0.6% sorbic acid, from 0% to 0.1% EDTA disodium, from 0 to 50 mM buffer, and a tonicity agent, with the remaining part of said composition being water, wherein said composition has a pH of from 4.5 to 5.5.

Another composition consists essentially of from 0.8 to 1.2% prednisolone acetate, from 20% to 25% hydroxypropyl-γ-cyclodextrin, from 0% to 0.12% hydroxypropylmethylcellulose, from 3 to 5 ppm polyhexamethylene biguanide HCl, from 0.1% to 0.6% sorbic acid, from 0 to 10 mM buffer, about 0.1% EDTA disodium, and a tonicity agent, with the remaining part of said composition being water, wherein said composition has a pH of about 4.8.

Example 1

Samples 1-20 were prepared having the components of Table 1 in addition to 1.2% Prednisolone Acetate, 25% Hydroxypropyl-gamma-cyclodextrin [Cavasol W8 HP, Wacker, Germany], 0.12% HPMC [Methocel, Dow Chemical Company, Midland, MI], 10 mM (pH 4.8) Acetic Acid/Na Acetate, and 0.1% EDTA in 100 Purified Water according to the following procedure.

Hydroxypropylmethylcellulose (HPMC) was slowly added to water at a temperature of 40°C with propeller mixing. The heat was removed, and mixing continued while the solution was allowed to cool to room temperature. All of the other excipients except HP-γ-cyclodextrin and prednisolone acetate were added to the HPMC solution or pure water, and the mixture was stirred

until all solids were completely dissolved. HP-γ-cyclodextrin (HPγCD) was added, and the mixture was stirred until the HPγCD was completely dissolved. Prednisolone acetate was added, and the mixture was stirred for a few minutes. The entire solution was autoclaved at 120°C for 20 minutes. Stirring continued at room temperature upon removing the solution from the autoclave. The pH was then adjusted by the addition of HCl and/or NaOH before addition of PHMB, and the solution was filtered through a 0.45 μm cellulose acetate membrane.

A brief description of the test procedure is as follows: *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 9027, *Escherichia coli* ATCC 8739, *Candida albicans* ATCC 10231 and *Aspergillus niger* ATCC 16404 were evaluated as the challenge organisms. For each organism, ten milliliters of product were dispensed into a polystyrene test tube. Sample tubes were then inoculated to contain approximately 1 x 10^5 to 1 x 10^6 colony-forming units (CFU) per mL of one of the five challenge organisms. Sample tubes were then vortexed and stored at 22.5 ± 2.5 °C. Standard 1-mL aliquots of each sample tube were assayed at 6 hours, 24 hours, 7 days, 14 days and 28 days to determine the numbers of viable CFU per mL. Removed aliquots were neutralized in Leethen broth followed by performing standard plate counts. *Candida albicans* and *Aspergillus niger* were not evaluated at 6 and 24 hours. The criteria for passing antimicrobial preservative effectiveness can be found in USP-NF and European Pharmacopoeias, and are summarized in Table 1a below.

Table 1a

USP, Ph Eur-A, and Ph Eur-B Antimicrobial Preservative Efficacy Test Criteria			
Organism	**USP**	**Ph Eur-A**	**Ph Eur-B**
S. aureus ATCC 6538 *P. aeruginosa* ATCC 9027 *E. coli* ATCC 8739	1.0 log at 7days 3.0 logs at 14 days	2 logs at 6 hours 3 logs at 24 hours No recovery at 28 days	1 log at 24 hours 3 logs at 7 days
C. albicans ATCC 10231 *A. niger* ATCC 16404	Stasis	2 logs at 7 days	1 log at 14 days

E. coli is **not** required to be evaluated by Ph Eur-A/B criteria. All criteria stipulate no increase after the required reductions.

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Table 1b

Sample	*S. aureus* ATCC 6538	*P. aeruginosa* ATCC 9027	*E. coli* ATCC 8739	*C. albicans* ATCC 10231	*A. niger* ATCC 16404
#1 2 ppm PHMB, 0.6% Boric Acid, 0.5 % Glycerol	Pass USP Fail Ph Eur-A Fail Ph Eur-B	Pass USP Fail Ph Eur-A Pass Ph Eur-B	Pass USP	Pass USP Pass Ph Eur-A Pass Ph Eur-B	Pass USP Fail Ph Eur-A Fail Ph Eur-B
#2 3 ppm PHMB, 0.6% Boric Acid, 0.5 % Glycerol	Pass USP Pass Ph Eur-A Pass Ph Eur-B	Pass USP Pass Ph Eur-A Pass Ph Eur-B	Pass USP	Pass USP Pass Ph Eur-A Pass Ph Eur-B	Pass USP Fail Ph Eur-A Pass Ph Eur-B
#3 3 ppm PHMB, No Boric Acid, 0.5 % Glycerol	Pass USP Pass Ph Eur-A Pass Ph Eur-B	Pass USP Pass Ph Eur-A Pass Ph Eur-B	Pass USP	Pass USP Pass Ph Eur-A Pass Ph Eur-B	Pass USP Fail Ph Eur-A Fail Ph Eur-B
#4 3 ppm PHMB, 0.6% Boric Acid, No Glycerol	Pass USP Pass Ph Eur-A Pass Ph Eur-B	Pass USP Pass Ph Eur-A Pass Ph Eur-B	Pass USP	Pass USP Pass Ph Eur-A Pass Ph Eur-B	Pass USP Fail Ph Eur-A Fail Ph Eur-B
#5 4 ppm PHMB, 0.6% Boric Acid, 0.5 % Glycerol	Pass USP Pass Ph Eur-A Pass Ph Eur-B	Pass USP Pass Ph Eur-A Pass Ph Eur-B	Pass USP	Pass USP Pass Ph Eur-A Pass Ph Eur-B	Pass USP Fail Ph Eur-A Fail Ph Eur-B
#6 5 ppm PHMB, 0.6% Boric Acid, 0.5 % Glycerol	Pass USP Pass Ph Eur-A Pass Ph Eur-B	Pass USP Pass Ph Eur-A Pass Ph Eur-B	Pass USP	Pass USP Pass Ph Eur-A Pass Ph Eur-B	Pass USP Fail Ph Eur-A Fail Ph Eur-B
#7 8 ppm PHMB, 0.6% Boric Acid, 0.5 % Glycerol	Pass USP Pass Ph Eur-A Pass Ph Eur-B	Pass USP Pass Ph Eur-A Pass Ph Eur-B	Pass USP	Pass USP Pass Ph Eur-A Pass Ph Eur-B	Pass USP Fail Ph Eur-A Fail Ph Eur-B
#8 10 ppm PHMB, 0.6% Boric Acid, 0.5 % Glycerol	Pass USP Pass Ph Eur-A Pass Ph Eur-B	Pass USP Pass Ph Eur-A Pass Ph Eur-B	Pass USP	Pass USP Pass Ph Eur-A Pass Ph Eur-B	Pass USP Fail Ph Eur-A Fail Ph Eur-B

Table 1.b (Continued) Summary of antimicrobial preservative effectiveness data

Sample	*S. aureus* ATCC 6538	*P. aeruginosa* ATCC 9027	*E. coli* ATCC 8739	*C. albicans* ATCC 10231	*A. niger* ATCC 16404
#9 2 ppm PHMB, 0.6% Sorbic Acid, 0.5 % Glycerol	Pass USP Fail Ph Eur-A Pass Ph Eur-B	Pass USP Pass Ph Eur-A Pass Ph Eur-B	Pass USP	Pass USP Pass Ph Eur-A Pass Ph Eur-B	Pass USP Pass Ph Eur-A Pass Ph Eur-B
#10 3 ppm PHMB, 0.6% Sorbic Acid, 0.5 % Glycerol	Pass USP Pass Ph Eur-A Pass Ph Eur-B	Pass USP Pass Ph Eur-A Pass Ph Eur-B	Pass USP	Pass USP Pass Ph Eur-A Pass Ph Eur-B	Pass USP Pass Ph Eur-A Pass Ph Eur-B
#11 3 ppm PHMB, No Sorbic Acid, No Glycerol	Pass USP Pass Ph Eur-A Pass Ph Eur-B	Pass USP Pass Ph Eur-A Pass Ph Eur-B	Pass USP	Pass USP Pass Ph Eur-A Pass Ph Eur-B	Pass USP Fail Ph Eur-A Fail Ph Eur-B
#12 3 ppm PHMB, 0.6% Sorbic Acid, No Glycerol	Pass USP Pass Ph Eur-A Pass Ph Eur-B	Pass USP Pass Ph Eur-A Pass Ph Eur-B	Pass USP	Pass USP Pass Ph Eur-A Pass Ph Eur-B	Pass USP Pass Ph Eur-A Pass Ph Eur-B
#13 4 ppm PHMB, 0.6% Sorbic Acid, 0.5 % Glycerol	Pass USP Pass Ph Eur-A Pass Ph Eur-B	Pass USP Pass Ph Eur-A Pass Ph Eur-B	Pass USP	Pass USP Pass Ph Eur-A Pass Ph Eur-B	Pass USP Pass Ph Eur-A Pass Ph Eur-B
#14 5 ppm PHMB, 0.6% Sorbic Acid, 0.5 % Glycerol	Pass USP Pass Ph Eur-A Pass Ph Eur-B	Pass USP Pass Ph Eur-A Pass Ph Eur-B	Pass USP	Pass USP Pass Ph Eur-A Pass Ph Eur-B	Pass USP Pass Ph Eur-A Pass Ph Eur-B
#15 8 ppm PHMB, 0.6% Sorbic Acid, 0.5 % Glycerol	Pass USP Pass Ph Eur-A Pass Ph Eur-B	Pass USP Pass Ph Eur-A Pass Ph Eur-B	Pass USP	Pass USP Pass Ph Eur-A Pass Ph Eur-B	Pass USP Pass Ph Eur-A Pass Ph Eur-B
#16 10 ppm PHMB, 0.6% Sorbic Acid, 0.5 % Glycerol	Pass USP Pass Ph Eur-A Pass Ph Eur-B	Pass USP Pass Ph Eur-A Pass Ph Eur-B	Pass USP	Pass USP Pass Ph Eur-A Pass Ph Eur-B	Pass USP Pass Ph Eur-A Pass Ph Eur-B
#17 100 ppm BAK, 0.6% Boric Acid, 0.5 % Glycerol	Pass USP Fail Ph Eur-A Fail Ph Eur-B	Pass USP Pass Ph Eur-A Pass Ph Eur-B	Pass USP	Pass USP Fail Ph Eur-A Pass Ph Eur-B	Pass USP Fail Ph Eur-A Pass Ph Eur-B
#18 150 ppm BAK, 0.6% Boric Acid, 0.5 % Glycerol	Pass USP Fail Ph Eur-A Fail Ph Eur-B	Pass USP Pass Ph Eur-A Pass Ph Eur-B	Pass USP	Pass USP Fail Ph Eur-A Pass Ph Eur-B	Pass USP Fail Ph Eur-A Pass Ph Eur-B
#19 200 ppm BAK, 0.6% Boric Acid, 0.5 % Glycerol	Pass USP Fail Ph Eur-A Fail Ph Eur-B	Pass USP Pass Ph Eur-A Pass Ph Eur-B	Pass USP	Pass USP Fail Ph Eur-A Pass Ph Eur-B	Pass USP Fail Ph Eur-A Pass Ph Eur-B
#20 200 ppm BAK, No Boric Acid, No % Glycerol	Pass USP Fail Ph Eur-A Fail Ph Eur-B	Pass USP Pass Ph Eur-A Pass Ph Eur-B	Pass USP	Pass USP Fail Ph Eur-A Fail Ph Eur-B	Pass USP Fail Ph Eur-A Pass Ph Eur-B

While not intending to be bound or limited in any way by theory, comparison of the data (Table 1b) for compositions 1-8 with that of

compositions 17-20 unexpectedly shows that 100-200 ppm of benzalkonium chloride (BAK) is significantly less effective than 3-10 ppm PHMB at preserving prednisolone acetate against *S. aureus*, as the BAK formulation failed both European tests. Similarly, the PHMB formulation is also clearly superior to the BAK formulation in preserving the formulation against *C. albicans*, as the PHMB formulation passed all of the tests, whereas the BAK formulation failed the Ph Eur-B tests. Thus, while not intending to limit the scope of the invention in any way, or be bound by theory, it appears that PHMB is superior to BAK in preserving ophthalmic compositions.

While not intending to limit the scope of the invention in any way, although PHMB is clearly superior to BAK overall in preserving ophthalmic formulations, it appears that PHMB is somewhat less effective than BAK in the case of *A. niger*. Surprisingly, the data for compositions 9-16 clearly shows the replacement of boric acid with sorbic acid corrects this deficiency, such that the PHMB/borate combination are effective against all of the tested pathogens in all of the tests when the concentration of PHMB is 3 ppm or greater.

CLAIMS

What is claimed is:

1. A composition comprising a steroid, a cyclodextrin, and polyhexamethylene biguanide.

2. The composition of claim 1 comprising a cyclodextrin selected from the group consisting of hydroxypropyl-β-cyclodextrin, hydroxypropyl-γ-cyclodextrin, sulfobutylether-β-cyclodextrin, and sulfobutylether-γ-cyclodextrin.

3. The composition of claim 1 wherein the steroid is selected from the group consisting of prednisolone, prednisone, androgens, estrogens, glucocorticoids, progestins, mineralocorticoids, and corticosteroids.

4. The composition of claim 1 wherein the steroid is selected from the group consisting of cortisone, hydrocortisone, prednisone, prednisolone, methylprednisone, triamcinolone, fluoromethalone, dexamethasone, medrysone, betamethasone, loteprednol, fluocinolone, flumethasone, mometasone, testosterone, methyltestosterone, and danazol.

5. The composition of claim 1 comprising sorbic acid.

6. The composition of claim 1 comprising prednisolone acetate.

7. The composition of claim 5 comprising prednisolone acetate.

8. The composition of claim 1 comprising from 0.1 ppm to 25 ppm PHMB HCl.

9. The composition of claim 1 comprising from 1 ppm to 5 ppm PHMB HCl.

10. The composition of claim 1 comprising from 3 ppm to 5 ppm PHMB HCl.

11. The composition of claim 8 comprising from 0.05% to 5% sorbic acid.

12. The composition of claim 9 comprising about 0.6% sorbic acid.

13. The composition of claim 1 wherein the concentration of the cyclodextrin is from 10% to 30%.

14. The composition of claim 1 wherein the pH is from 4 to 6.

15. The composition of claim 6 wherein the pH is from 4.5 to 5.5.

16. The composition of claim 6 wherein the pH is about 4.5.

17. The composition of claim 1 consisting essentially of from 0.6 to 1.8% prednisolone acetate, from 10% to 25% hydroxypropyl-γ-cyclodextrin, from 0% to 0.25% hydroxypropylmethylcellulose, from 3 to 10 ppm PHMB HCl, from 0.05% to 0.6% sorbic acid, from 0% to 0.1% EDTA disodium, from 0 to 50 mM buffer, and a tonicity agent, with the remaining part of said composition being water, wherein said composition has a pH of from 4.5 to 5.5.

18. The composition of claim 1 consisting essentially of from 0.8 to 1.2% prednisolone acetate, from 20% to 25% hydroxypropyl-γ-cyclodextrin, from 0% to 0.12% hydroxypropylmethylcellulose, from 3 to 5 ppm PHMB HCl, from 0.1% to 0.6% sorbic acid, from 0 to 10 mM buffer, about 0.1% EDTA disodium, and a tonicity agent, with the remaining part of said composition being water, wherein said composition has a pH of about 4.8.

19. A method comprising providing an ophthalmic composition comprising a steroid and cyclodextrin with an effective amount of a polyhexamethylene biguanide, wherein said method prevents, attenuates, or reduces the pathogenic contamination of said composition.

20. The method of claim 19 wherein said steroid is prednisolone acetate.

INTERNATIONAL SEARCH REPORT

Inte I Application No
PCT/US2005/014612

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K9/00 A61K31/00 A61K31/155 A61K47/48 A61K47/18 A61K47/40

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, PAJ, BIOSIS, MEDLINE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0 958 836 A (MENICON CO., LTD) 24 November 1999 (1999-11-24) paragraphs '0007!, '0028!; claims 1-6	1-18
A	EP 0 579 435 A (LOFTSSON, THORSTEINN; CYCLOPS H.F) 19 January 1994 (1994-01-19) page 1 - page 5; claims 1-13	1-18
A	US 5 998 488 A (SHINOHARA ET AL) 7 December 1999 (1999-12-07) cited in the application page 1; claims 1-6	1-18

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
30 September 2005	07/10/2005

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Kardas-Llorens, E

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INTERNATIONAL SEARCH REPORT

Int. I Application No
PCT/US2005/014612

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	PATENT ABSTRACTS OF JAPAN vol. 009, no. 309 (C-318), 5 December 1985 (1985-12-05) & JP 60 149530 A (TAKEDA YAKUHIN KOGYO KK), 7 August 1985 (1985-08-07) cited in the application abstract	1-18
A	US 5 576 311 A (GUY ET AL) 19 November 1996 (1996-11-19) claims 1-25	1-18
A	EP 0 306 455 A (WARNER-LAMBERT COMPANY) 8 March 1989 (1989-03-08) pages 1,2; claims 1-13	1-18

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure. If the application proceeds into the regional phase before the EPO, the applicant is reminded that a search may be carried out during examination before the EPO (see EPO Guideline C-VI, 8.5), should the problems which led to the Article 17(2) declaration be overcome.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2005/014612

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter... al Application No
PCT/US2005/014612

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